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# Applications of surface-enhanced Raman scattering (SERS) for biomolecules

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**Abstract :** Surface-enhanced Raman Scattering (SERS) is a new technique for studying the biomolecules. The SERS signals are found to be enhanced enormously over the normal Raman signals. A very lower concentration of the sample is required. Some experimental techniques of SERS have been discussed in the present communication.

**Keywords :** Raman spectroscopy, surface-enhanced Raman, SERS, adsorption, surface processes.

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## 1. Introduction

Raman spectroscopy is an important technique for chemical and biological analysis because of its excellent capability of chemical group identification. But a major limitation of the conventional Raman method is its low sensitivity. Recently the Raman technique has enjoyed an increased interest among the spectroscopists following the observations of giant Raman enhancement for molecules adsorbed on special metal surfaces or on metal colloids exhibit inordinate Raman signals whose intensity is $\approx 10^6 - 10^7$ times larger than the normal Raman signals. This enormous increase in Raman signal arises from a surface enhancement process and hence the phenomenon has been termed as Surface-Enhanced Raman Scattering (SERS).

Raman spectroscopy is widely used in the study of biomolecules ; but a major disadvantage of normal Raman technique is the small scattering cross section of the sample because of which very weak Raman signals come out. Another culprit which hinders the Raman study of biomolecules is the strong fluorescence background. The newly discovered method, SERS, has a great advantage over these difficulties as it enhances the weak signals enormously and suppresses the fluorescence. The present communication will point out some recently developed techniques for Surface-Enhanced Raman (SER) study of biomolecules.

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## 2. Techniques

*(i) Metal electrodes :*

Since the first observations of SERS by molecules adsorbed on silver electrodes (Jeanmaire and Van Duyne 1977, Albrecht and Creighton 1977) many studies have been conducted on compounds adsorbed at the surface of metal electrode (Chang and Furtak 1982). Electrochemical cells for SERS studies generally employed silver electrodes, although SER studies have also been conducted using other metal electrodes (Pettinger *et al* 1980, Fleischmann *et al* 1985, Loo 1983). The electrolyte of the electrochemical cell contains the molecules under study.

The intensity of Raman scattering is strongly dependent on the state of roughness of the metal surface. The early experiments and much subsequent work on SERS has been carried out on silver electrodes randomly roughened by an electrochemical oxidation-reduction cycle in aqueous electrolysis. The working electrode is generally placed in a position such that the laser excitation can be focused onto its surface, and the Raman scattered light can be efficiently collected by appropriate optics. Strong SERS signals from the adsorbed molecules on roughened surface appear only after electrochemical oxidation-reduction cycles.

*(ii) Colloidal metal particles :*

The metal electrode technique of SERS is less favourable for probing the physics of enhancement, since it is difficult to measure some of their other optical properties, particularly their absorption spectra, and to account for these properties scientists drew their attention to more regular finely divided metal surfaces, viz. colloidal dispersions or evaporated island film, or the surface of a diffraction grating. The study of SERS on colloidal metal particles bears a significant role in proving the first clear demonstration that the enhancement in SERS is associated with the resonant excitation of electron density oscillations in metal surface.

Colloidal solution is prepared by reducing a dissolved metal salt by means of an appropriate reducing agent in either an aqueous or non-aqueous medium. Surface-enhanced Raman scattering by colloidal metal particles was first reported by Creighton *et al* (1979), who made investigation of SERS on pyridine adsorbed on aqueous silver and gold colloids. Silver colloids are generally prepared by rapidly mixing a $1.0 \times 10^{-3}$ M aqueous solution of $AgNO_3$ with $2.0 \times 10^{-3}$ M aqueous solution of ice-cold $NaBH_4$, at the ratio of 1 : 3 by volume.

*(iii) Metal island films :*

Thin Ag island films are prepared by evaporating the metal onto a glass or saphire substrate in a vacuum of $2 \times 10^{-7}$ torr, at a rate of 0.2 to 0.4 Å per second. The mass thickness of the film was monitored by a quartz crystal oscillator mounted close to the sample substrate. The films were then removed from the vacuum, immersed then momentarily in an aqueous solution of molecules to be studied, rinsed with distilled water and air-dried.

(iv) *Silver-coated microsphere substrates :*

In general, a 50-$\mu$l volume of a suspension of latex or Teflon submicron spheres was applied to the surface of the substrate. The different types of substrates investigated include filter paper, cellulosic membranes, glass plates, or quartz materials (Alak and Vo-Dinh 1988, Moody *et al* 1987). The substrate was then placed on a high-speed spinning device and spun at 800-2000 rpm for about 20 s. The silver was deposited on the microsphere-coated substrate in a vacuum evaporator at a deposition rate of 2 nm/s. The thickness of the silver layer deposited was generally 50-100 nm.

(v) *Silver-coated fumed silica substrate :*

Fumed silica-based substrate is quite SERS active and easy to prepare. Fumed silica has been used as a thickening agent in various industrial processes, including coating and cosmetics preparations. In the preparation of SERS materials, the selection of the appropriate types of fumed silica is important. Fumed silica is manufactured in different grades, which vary with respect to surface area, particle diameter, and degree of compression. The fumed silica particles were suspended in a 10% water solution and were then coated onto a glass plate or filter paper. The substrate was then coated with a 50-100 nm layer of silver by thermal evaporation. With these types of substrates, the fumed silica materials, which have submicron-size structures, provide the rough-surface effect for the SERS process.

(vi) *Silver-coated quartz posts :*

The preparation of $SiO_2$ prolate posts is a multistep operation that involves plasma etching of $SiO_2$ with a silver island film as an etch mask (Chang and Futak 1982, Enlow *et al* 1986). Since fused quartz etches much more slowy than thermally deposited quartz, a 500 nm layer of $SiO_2$ was first thermally evaporated onto fused quartz at a rate of 0.1-0.2 nm/s. The resulting crystalline quartz was annealed to the fused quartz for 45 minute at approximately 950°C. A 5-nm silver layer was then evaporated into the thermal $SiO_2$ layer, and the substrate was flash-heated for 20 s at 500°C. This heating causes the thin silver layer to bead up into small globules, which act as etch masks. The substrate was then etched for 30-60 minute in $CHF_3$ plasma to produce submicron prolate $SiO_2$ posts, which were then coated with an 80 nm layer of silver at normal evaporation angle.

(vii) *Metal-coated cellulose substrates :*

Certain types of micropore filter papers coated with a thin layer of evaporated silver appear to provide efficient SERS-active substrates. Scanning electron micrographs of these cellulosic materials showed that these surfaces consist of fibrous 10-$\mu$m strands with numerous tendrils that the necessary microprotrusions required for the SERS enhancement. The simple technique employing

silver-coated cellulose substrate was used to analyze benzo [a] pyrene DNA adducts (Vo-Dinh *et al* 1987). The carcinogenic activity of benzo [a] pyrene (BP) in animals and its metabolic activation to the ultimate carcinogenic metabolite, BP-7, 8-diol-9,10-epoxide (BPDE) have been well established (Girimmer 1983).

*(viii) Silver membrane :*

One of the simpler types of solid substrates that have been investigated by Vo-Dinh *et al* (1987) is the silver membrane used for air particulate sampling. The filter already has micropores that provide the microstructure required to induce SERS. These substrates consist of silver membranes and can, therefore, be used directly as SERS-active substrates without requiring silver coating. The silver membrane substrate was used directly as SERS substrate without requiring any chemical treatment or metal evaporation. The SERS spectrum of 1-nitropyrene obtained with the silver membrane is similar to that observed with the microsphere-coated substrates or the prolate quartz post substrates reported previously.

*(ix) Chemically etched metal surfaces :*

Miller *et al* (1984) described two simple etching procedures used to produce SERS active copper surfaces. In the first procedure, copper foil was etched for 40 minute in 2 mol $cm^{-3}$ nitric acid at room temperature. The second procedure consisted of sandblasting copper foil with $Al_2O_3$ at 4 bar pressure and subsequently etching for 2 minute. An electron scanning microscope procedures could produce surface roughness on the 10-100 nm scale. These structures produce large electromagnetic fields on the surface when the incident photon energy is in resonance with localized surface plasmons.

## 3. Conclusions

In the study of biomolecules the SERS method is very effective. It enhances the Raman signals enormously without enhancing the fluorescence background. Moreover, most of the biochemicals are hazardous in nature. To avoid environmental pollution during the investigation of those samples, very lower concentrations should be used. In fact, for SERS study, there is no need of substantial amount of the sample. Even a lower concentration down to $10^{-4}$ M is sufficient to record the surface-enhanced Raman (SER) spectra.

**References**

Alak A and Vo-Dinh T 1988 *Anal. Chim. Acta* **206** 333
Albrecht M G and Creighton J A 1977 *J. Am. Chem. Soc.* **99** 5215
Chang R K and Furtak T E (eds) 1982 *Surface-Enhanced Raman Scattering* (New York : Plenum)
Creighton J A Blatchford C B and Albrecht M G 1979 *J. Chem. Soc. Faraday Trans. II* **75** 790
Enlow P D, Buncick M C, Warmack R J and Vo-Dinh T 1986 *Anal. Chem.* **58** 1119
Fleischmann M, Graves P R and Robinson J 1985 *J. Electroanal. Chem.* **182** 87

Girimmer 1983 *Ed. Environmental Carcinogens : Polycyclic Aromatic Hydrocarbons* (Boca Raton: CRC press)
Jeanmaire D J and Van Duyne R P 1977 *J. Electroanal. Chem.* **84** 1
Loo B H 1983 *J. Phys. Chem.* **87** 3003
Miller S K, Baiker A, Meier M and Wokaun A 1984 *J. Chem. Soc. Faraday Trans. I* **80** 1305
Moody R L, Vo-Dinh T and Fletcher W H 1987 *Appl. Spectrosc.* **41** 966
Pettinger B, Wenneng U and Wetzel H 1980 *Surf. Sci.* **101** 409
Vo-Dinh T, Utzel M and Morrison A 1987 *Appl. Spectrosc.* **41** 605